Uptake Kinetics of Arsenic Species in Rice Plants

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Arsenic (As) finds its way into soils used for rice (*Oryza sativa*) cultivation through polluted irrigation water, and through historic contamination with As-based pesticides. As is known to be present as a number of chemical species in such soils, so we wished to investigate how these species were accumulated by rice. As species found in soil solution from a greenhouse experiment where rice was irrigated with arsenate contaminated water were arsenite, arsenate, dimethylarsinic acid, and monomethylarsonic acid. The short-term uptake kinetics for these four As species were determined in 7-d-old excised rice roots. High-affinity uptake (0–0.0532 mM) for arsenite and arsenate with eight rice varieties, covering two growing seasons, rice var. Boro (dry season) and rice var. Aman (wet season), showed that uptake of both arsenite and arsenate by Boro varieties was less than that of Aman varieties. Arsenite uptake was active, and was taken up at approximately the same rate as arsenate. Greater uptake of arsenite, compared with arsenate, was found at higher substrate concentration (low-affinity uptake system). Competitive inhibition of uptake with phosphate showed that arsenite and arsenate were taken up by different uptake systems because arsenate uptake was strongly suppressed in the presence of phosphate, whereas arsenite transport was not affected by phosphate. At a slow rate, there was a hyperbolic uptake of monomethylarsonic acid, and limited uptake of dimethylarsinic acid.

Groundwater contamination by As has been reported from many countries, with the most severe problems occurring in Asia, namely Bangladesh (Dhar et al., 1997; Biswas et al., 1998; Nickson et al., 1998; Chowdhury et al., 1999), West Bengel India (Mandal et al., 1996; Mandal et al., 1997), China (Huang et al., 1992; Liangfang and Jianghong, 1994), and Taiwan (Smith et al., 1992; Chen et al., 1995). In Bangladesh, groundwater is the primary source of drinking water for up to 90% of a total population of 130 million (World Health Organization [WHO], 2001). In some areas of Bangladesh, groundwater As concentrations reach 2 mg L^{-1} (Tondel et al., 1999; British Geological Survey, 2000), where the WHO provisional guideline value for drinking water is only 0.01 mg L^{-1}. The national standard for drinking water in Bangladesh is 0.05 mg L^{-1}. According to the British Geological Survey (2000), in tube wells from 41 of the total 64 districts in Bangladesh, 51% of the samples were above 0.01 mg L^{-1} (WHO-permissible limit for drinking water), 35% were above 0.05 mg L^{-1}, 25% were above 0.10 mg L^{-1}, 8.4% were above 0.3 mg L^{-1}, and 0.1% were above 1.0 mg L^{-1}. An estimated population of 25 million are exposed to As concentrations of more than 0.05 mg L^{-1} (Bangladesh-permissible limit), and the number would be approximately doubled if WHO limit of 0.01 mg L^{-1} were adopted (School of Environmental Studies and Dhaka Community Hospital, 2000). It is estimated that As in drinking water will cause 200,000 to 270,000 deaths from cancer in Bangladesh alone (WHO, 2001). The people of this region are not just drinking the contaminated groundwater, but also using this water for crop irrigation. In Bangladesh, irrigation is mostly dependent on groundwater. Presently, 75% of the total cropped area and 83% of the total irrigated area are used for rice (*Oryza sativa*) cultivation (Dey et al., 1996). Background levels of As in soils are 4 to 8 mg As kg^{-1}. In areas irrigated with contaminated water, the soil level can reach up to 83 mg As kg^{-1} (Ullah, 1998). Another report recorded elevated As concentrations of up to 57 mg As kg^{-1} in soils collected from four districts of Bangladesh (Alam and Sattar, 2000).

Inorganic As is the predominant form of As in soil (Johnson and Hiltbold, 1969) and in ground water (Samanta et al., 1999). Under aerobic soil conditions, arsenate dominates, whereas in submerged soil condition the predominant species is arsenite (Masscheleyn et al., 1991; Marin et al., 1993a; Onken and Hossner, 1995, 1996). There is evidence of As methylation in paddy soil systems, where inorganic species were converted to organic form by microorganisms (Takamatsu et al., 1982). Arsenate was found as the major component, with lower levels of arsenite, monomethylarsonic acid (MMAA), and dimethylarsinic acid (DMAA). We investigated the conversion of arsenate to other species in the paddy soil in the study reported here.

There are a number of studies investigating the mechanism of As uptake by different plant species (Asher and Reay, 1979; Meharg and Macnair, 1992a,

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1992b; Meharg et al., 1994), but little work has been done on the uptake mechanism of As species in rice. Also, our knowledge of the kinetics of uptake of organic As species and for arsenite for plants is poorly understood in general. Arsenate uptake by a range of plants is via high-affinity phosphate transporter because arsenate and phosphate are analogous (Jung and Rothstein, 1965; Asher and Reay, 1979; Beever and Burns, 1980; Silver and Misra, 1988; Ullrich-Eberius et al., 1989; Meharg and Macnair, 1990, 1992a). It is generally thought that uptake of organic As species is lower than inorganic species (Odanaka et al., 1987). However, Marin et al. (1992, 1993b) found high uptake of organic species (MMAA and DMAA) when rice plants were treated with salts of these species in hydroponic culture. Uptake kinetic studies were conducted with arsenate, arsenite, MMAA, and DMAA to observe how these species are taken up into the plants. To investigate if there was any variation in uptake of two inorganic As species (arsenite and arsenate) by different rice varieties, we studied uptake kinetics in eight varieties that are grown in two rice-growing seasons of Bangladesh, rice var. Boro (dry season) and rice var. Aman (wet season).

RESULTS

As Species in Soil Solution

The As species found in soil solution of rice rhizosphere grown under flooded paddy conditions and irrigated with arsenate contaminated (0–0.1064 mM) solutions were arsenite, arsenate, DMAA, and MMAA. In general, arsenite was the most predominant species, followed by DMAA, arsenate, and MMAA (Fig. 1). Concentrations of arsenite, arsenate, DMAA, and MMAA ranged between approximately 36% and 63%, 1% and 39%, 11% and 44%, and 0% and 14%, respectively. DMAA accounts for 25% to 44% of the total As species for treatments ≤ 0.0532 mM.

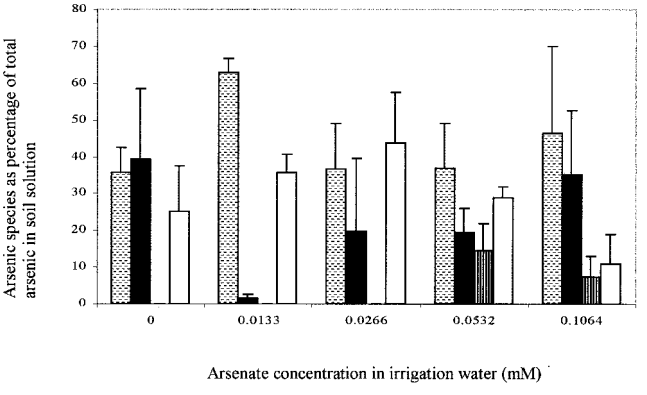

Figure 1. As species present in soil solution from a greenhouse experiment when rice was irrigated with arsenate solutions. ▨, Arsenite; ■, arsenate; ▥, MMAA; □, DMAA. Error bars represent ±SE of three replicates.

Table I. *Total As concentrations in soil solution used for speciation study and total soil As concentration after termination of irrigation treatments*

Each value is the mean of three replications with ±SE.

Arsenate Concentration in Irrigation Water	Arsenic Concentration in the Soil Solution	Total Arsenic Concentration in Soils
mg L⁻¹	*μg L⁻¹*	*mg kg⁻¹*
0	8 ± 0.3	31 ± 1
1	20 ± 0.7	51 ± 3
2	34 ± 5	64 ± 2
4	38 ± 8	95 ± 3
8	321 ± 184	197 ± 4

Thereafter, the proportion of DMAA was reduced to 11% at the highest arsenate treatment (0.1064 mM). MMAA was recorded only in the two highest arsenate treatments, constituting about 14% and 7% of the total As species, respectively, for 0.0532 and 0.1064 mM arsenate treatments. The proportion of organic species (DMAA plus MMAA) was lowest (18%) in the highest arsenate treatment (0.1064 mM). This reduction in the proportion of organic species in the highest arsenate treatment might be because of higher concentrations of As in soil solution, which may inhibit growth of microorganisms responsible for methylation of inorganic species in soil. Error assessments with percentage As speciation were large, representing heterogeneity in production of As speciation between replicates. The total concentrations of As in soil solution used in the speciation study and the concentration of As in soil at termination of the experiment are presented in Table I.

High-Affinity Uptake Kinetics of Arsenite and Arsenate by Different Rice Varieties

Arsenite and arsenate influx in all varieties showed a hyperbolic increase with increasing concentrations of arsenite and arsenate (Fig. 2). For both inorganic As species, the concentration dependent influx data fit better to Michaelis-Menten functions than to linear regressions (Table II). In the case of arsenite influx, the mean R^2 values in rice var. Aman were 0.990 and 0.782, and in rice var. Boro 0.976 and 0.747 for Michaelis-Menten and linear regression, respectively. For arsenate, mean R^2 values in rice var. Aman were 0.972 and 0.549, and in rice var. Boro 0.954 and 0.497 for Michaelis-Menten and linear regression, respectively. Thus, it was concluded that uptake of both species was hyperbolic rather than linear. The kinetics parameters for both the As species differ considerably between Aman and Boro season varieties. The average V_{max} for arsenite uptake was 175.0 ± 33.9 and 120.3 ± 5.7 nmol g^{-1} fresh weight h^{-1} for Aman and Boro season varieties, respectively, and for arsenate, 132.9 ± 13.4 and 97.0 ± 10.3 nmol g^{-1} fresh weight h^{-1} (Table II). The average K_m for arsenite uptake was 0.0229 ± 0.0103 and 0.0155 ± 0.0027

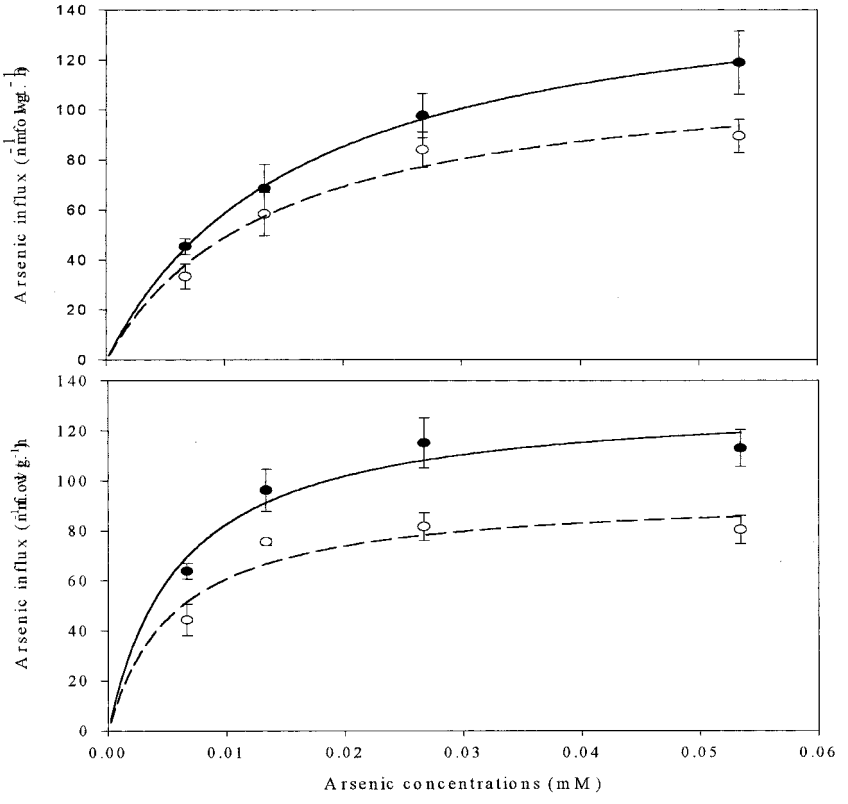

Figure 2. Concentration-dependent kinetics for high-affinity root arsenite and arsenate influx for eight rice varieties (four from Boro season ○, dashed line; and four from Aman season ●, solid line). Each point is the average of four varieties (each variety is the average of three replicates) and error bars are ±SE of the mean of four varieties

(mM), and for arsenate uptake 0.0059 ± 0.0012 and 0.0063 ± 0.0026 (mM), for Aman and Boro season varieties, respectively (Table II). Aman varieties have higher V_{max} for both the species and higher K_m for arsenite and lower K_m for arsenate, compared with Boro varieties (Table II). Overall, the V_{max} is higher for arsenate, whereas the K_m is lower for arsenate.

High- and Low-Affinity Uptake of Arsenite and Arsenate in Rice

There are two uptake systems for both arsenate and arsenite present in the roots of rice var. BR11, described by additive Michaelis-Menten functions: One system dominates at lower substrate concentrations (high-afinity uptake system) and another one at high substrate concentrations (low-affinity uptake system). Both carriers obey saturation kinetics. Concentration-dependent influx isotherms for both arsenite and arsenate fit well (R^2 = 0.9997 and 0.9980 for arsenite and arsenate, respectively) to an additive Michaelis-Menten function (Fig. 3). The high-affinity V_{max} and K_m values were 88.8 and 0.0039, respectively, for arsenite and 161.3 and 0.0157, respectively, for arsenate. The V_{max} and K_m values for arsenate are similar to the high-affinity uptake kinetic parameters presented in Table II, but those for arsenite are lower. The low-affinity V_{max} and K_m values both for arsenite and arsenate are extremely high, can be considered unrealistic, and might be because of the fact that at higher substrate concentrations the influx data fit better to a linear model rather than a nonlinear one. Over the concentration range of 0 to 0.25 mM, both arsenate and arsenite uptake rates were comparable; thereafter, arsenate uptake was considerably less than arsenite.

As Influx at Different Phosphate Concentrations

The uptake rate at 0.05 mM arsenate decreased significantly ($P < 0.001$) with increasing phosphate concentration present in the incubating solution (Fig. 4). Highest arsenate influx of 171.2 nmol g^{-1} fresh weight h^{-1} was found in the treatment where no phosphate was present in the incubating solution, which decreased by 9%, 30%, 53%, 66%, 80%, and 88% at 0.01, 0.025, 0.05, 0.1, 0.25, and 0.5 mM phosphate treatment, respectively. Uptake rate of 0.05 mM arsenite concentration on the other hand was independent of phosphate concentration (Fig. 4).

DMAA and MMAA Uptake by Rice

DMAA uptake was poorly described by Michaelis Menten kinetics (R^2 = 0.673), and by a linear function (R^2 = 0.584; Fig. 5). MMAA uptake, on the other hand showed, a hyperbolic increase with increasing MMAA concentration and fitted well to a Michaelis-Menten function (R^2 = 0.997) (Fig. 5). DMAA and MMAA have much lower rates of uptake than arsenite and arsenate (Fig. 5). At the substrate concentration of 0.0533 mM arsenite, arsenate, MMAA, and

Table II. *Kinetic parameters for arsenite and arsenate influx in eight rice varieties representing two rice growing seasons, Aman (rice var. 1, BR11; rice var. 2, BR23; rice var. 3, BRRI Dhan 31, and rice var. 4, BRRI Dhan 33) and Boro (rice var. 5, BR1; rice var. 6, BRRI Dhan 26; rice var. 7, BRRI Dhan 27, and rice var. 8, Purbachi)*

Kinetic parameters were calculated from mean As influx ($n = 3$) using Michaelis-Menten function (nonlinear regression) and linear regression model.

Variety and Season	Nonlinear Regression			Linear Regression		
	V_{max}	K_m	R^2	a	b	R^2
	nmol g^{-1} fresh weight	*mM*				
Arsenite, Aman						
V1	213.3	0.0223	0.995	27.32	2,582	0.856
V2	244.9	0.0521	0.984	13.01	2,214	0.946
V3	141.4	0.0110	0.989	31.81	1,884	0.739
V4	100.4	0.0061	0.991	32.35	1,306	0.585
Average ± SE	175.0 ± 33.0	0.0229 ± 0.0103		26.12 ± 4.51	1,997 ± 271	
Arsenite, Bo-o						
V5	118.4	0.0123	0.941	25.31	1,516	0.652
V6	130.1	0.0098	0.992	32.07	1,714	0.691
V7	105.1	0.0187	0.973	15.87	1,306	0.791
V8	127.6	0.0213	0.997	16.97	1,561	0.852
Average ± SE	120.3 ± 5.7	0.0155 ± 0.0027		22.56 ± 3.81	1,524 ± 84	
Arsenate, Aman						
V1	154.2	0.0070	0.960	47.42	1,964	0.556
V2	141.9	0.0074	0.990	41.50	1,855	0.623
V3	141.7	0.0068	0.947	44.45	1,783	0.531
V4	93.72	0.0024	0.991	40.31	1,230	0.486
Average ± SE	132.9 ± 13.4	0.0059 ± 0.0012		43.42 ± 1.59	1,708 ± 164	
Arsenate, Bo-o						
V5	100.2	0.0047	0.957	37.54	1,221	0.461
V6	91.8	0.0049	0.979	33.00	1,159	0.512
V7	73.3	0.0018	0.945	36.03	814.8	0.316
V8	122.8	0.0139	0.934	23.16	1,593	0.698
Average ± SE	97.0 ± 10.3	0.0063 ± 0.0026	–	32.43 ± 3.23	1,197 ± 159	–

DMAA, the uptake rates are 147, 126, 12.7, and 5.7 nmol g^{-1} fresh weight h^{-1}, respectively. The high-affinity kinetics parameters also show considerable difference in uptake among the species. V_{max} for arsenite, arsenate, and MMAA were 213.3, 154.2, and 15.43 nmol g^{-1} fresh weight h^{-1}, respectively.

DISCUSSION

The soil solution speciation study revealed that the most predominant forms of As in soil solution were inorganic and constituted about 56% to 82% of the total (Fig. 1). In the paddy soil environment, the applied arsenate was readily converted to arsenite. The presence of arsenite in the soil solution at a larger proportion corroborates with the results of a number of investigators (Masscheleyn et al., 1991; Marin et al., 1993a; Onken and Hossner, 1995, 1996) who found arsenite as the predominant species in the submerged soil environment. Considerable quantities of DMAA and smaller amounts of MMAA were present in the soil solution (Fig. 1), confirming that microbiological transformation of inorganic species to organic form occurs in the paddy soil (Takamatsu et al., 1982). This transformation to organic form is beneficial because of the lower toxicity of organic species (Fowler, 1977; National Research Council of Canada, 1978). Besides, there are reports of As loss from soil through volatilization of methylated arsines that would potentially diminish the concentration of As to which plants would be exposed (Cullen and Reimer, 1989). Onken and Hossner (1995), in their greenhouse pot experiment with rice, reported a loss of As from soil solution through volatilization. Woolson (1977) measured 1% to 18% loss of As as dimethyl- and trimethylarsine from soil depending on the arsenical compounds added to the soil. We also calculated a loss of As of up to 23% from the paddy soil in a greenhouse experiment (M.J. Habedin, unpublished data), which supports other evidence of As volatilization from soil.

We studied high-affinity uptake kinetics of arsenite and arsenate with four Aman and four Boro varieties that are recommended for two different agro-ecosystems in Bangladesh. Aman varieties are cultivated during July through December. In general, no irrigations, or one to two supplemental irrigations at the later stage of the crop growth, are required for Aman rice because monsoon rains fall during July to October. Boro varieties, on the other hand, are cultivated during December/January to May (when little or no rainfall occurs) and generally have complete

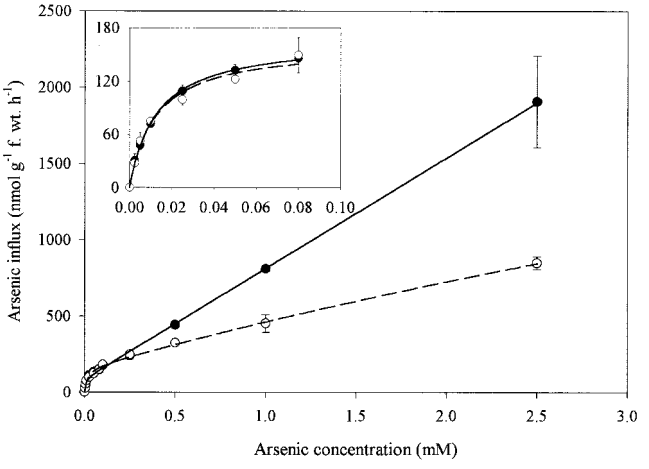

Figure 3. Concentration dependent kinetics for high- and low-affinity root arsenite (●, solid line) and arsenate (○, dashed line) influx of Aman rice var. BR11. Each point is an average of three replicates. Error bars are ±SE of the replicates. Insert depict the kinetics of arsenite and arsenate at lower substrate concentration (0–0.08 mM).

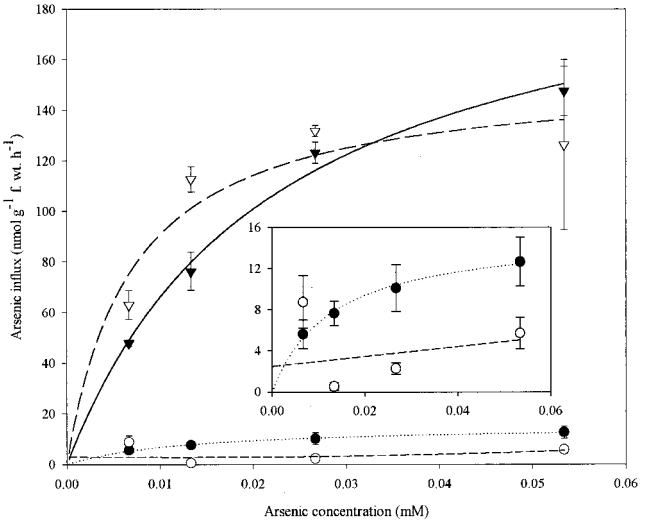

Figure 5. Concentration-dependent kinetics for high-affinity root arsenite (▼, solid line) arsenate (▽, long dashed line), DMAA (○, short dashed line), and MMAA (●, dotted line) influx for an Aman rice var. BR11. Each point is an average of three replicates. Error bars are the ±SE of the replicates. Insert, Uptake of MMAA and DMAA.

dependence on groundwater irrigation. The lower values of V_{max} for both arsenite and arsenate in Boro varieties compared with Aman (Table I) suggest a distinct difference in uptake of these two inorganic As species between the two rice season varieties. However, comparable values of K_m in Boro and Aman season varieties for both the species indicate no varietal difference in affinity for arsenite and arsenate over the high-affinity range. The higher uptake rate of arsenite and arsenate in Aman rice varieties (Fig. 2) might be because of varietal differences in some physiological or morphological attributes of the root systems. The physiological attributes include concentration of transporters in the plasma membrane, and the morphological attributes include root length, root diameter, and root hairs. Greater length and smaller diameter of roots will result in a higher surface area per unit mass of roots, and can cause higher uptake compared with the root mass having lower surface area. Higher K_m values for arsenite compared with arsenate suggest a lower affinity of the arsenite carrier, despite its higher uptake. Uptake kinetics characteristics can be considered one of the important criteria for selecting a variety to use in areas irrigated by As-contaminated irrigation water.

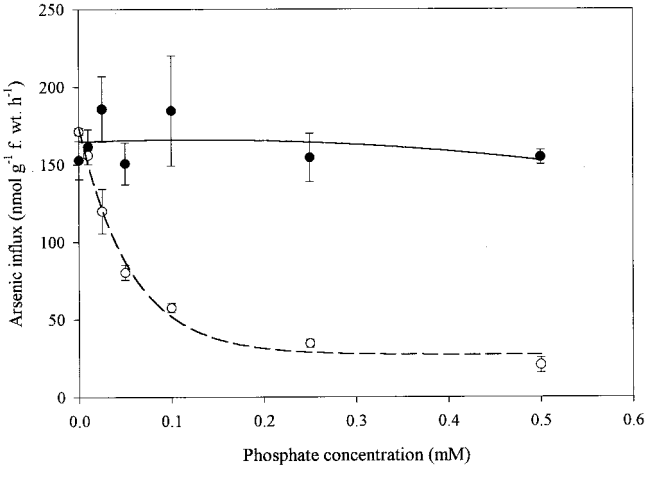

Figure 4. Uptake of 0.05 mM arsenite (●, solid line) and 0.05 mM arsenate (○, dashed line) by an Aman rice var. BR11 at different concentrations of phosphate (0–0.5 mM). Error bars are ±SE of three replicates.

There are a number of studies investigating uptake kinetics of arsenate, both in higher and lower plants (Asher and Reay, 1979; Lee, 1982; Meharg and Macnair, 1992a, 1992b; Meharg et al., 1994). In general, arsenite uptake kinetics have not been described for higher plants. A better fit of arsenite and arsenate uptake to a Michaelis-Menten model compared with a linear regression model suggests that transport of these two inorganic species is an active process, which requires an energy supply as a driving force, and selective binding sites. We observed comparable uptake for both arsenite and arsenate at lower concentrations, and much higher uptake of arsenite at higher concentrations. This is in contrast with the comparative uptake rate studies for arsenite and arsenate in the ericoid mycorrhizal fungus (*Hymenoscyphus ericae*) reported by Sharples et al., (2000). They found 3- to 4-fold less uptake rate of arsenite than arsenate over the high-affinity range (0.01 mM), and 15-fold less uptake rate of arsenite than arsenate over the low-affinity range (0.75 mM). High uptake rates of arsenite by rice is a matter concern because it is the dominant As species in the highly reduced rice soil environment, as illustrated in the data presented in Figure 1. Although arsenite was actively taken up by

rice plants in our studies, the nature of transporter involved is not clear. Lee (1982) showed that there are a number of analogs to major nutrient ions with respect to transport across the plasma membrane. Wysocki et al. (2001) were the first to characterize the molecular mechanism of arsenite uptake in eukaryotes, showing that arsenite was transported across the plasma membrane of *Saccharomyces cerevisiae* via a glycerol channel protein. The mechanism utilized by higher plants has yet to be determined.

Uptake of As at 0.05 mM arsenite and arsenate (i.e. high-affinity uptake) with different concentrations of phosphate showed that arsenite uptake was not inhibited by phosphate, even at high phosphate concentrations; this was in contrast to arsenate, where the presence of phosphate strongly inhibited the uptake. This result for arsenate is in full agreement with the previous studies on barley (*Hordeum vulgare*; Asher and Reay, 1979; Lee, 1982), *Holcus lanatus* (Meharg and Macnair, 1992a, 1992b), and *Deschampsia cespitosa* (Meharg and Macnair, 1994). Our uptake kinetics results for arsenite and arsenate with different concentrations of phosphate were also supported by Tsutsumi (1983), who found no significant change in rice arsenite toxicity when seedlings were exposed to different concentrations of phosphate, but did observe reduced arsenate toxicity with increased phosphate concentrations. Thus, in flooded soil environments where arsenite is the predominant species (Masscheleyn et al., 1991; Marin et al., 1993a; Onken and Hossner, 1995, 1996), phosphate application would not inhibit As toxicity and uptake. There are also discrepancies regarding the effectiveness of phosphate in reducing arsenate toxicity under field conditions (Jacobs and Keeney, 1970; Woolson et al., 1973; Creger and Peryea, 1994). This discrepancy arises because most of the experiments showing phosphate was an inhibitor of arsenate uptake were conducted on plants growing hydroponically, rather than in soil. In soils, added phosphate displaces the sorbed arsenate from exchange sites and therefore increases the solubility, phyto-availability, and movement down the soil profile of arsenate (Davenport and Peryea, 1991; Peryea, 1991; Peryea and Kammereck, 1997; Qafoku et al., 1999).

Because the most predominant As species in the soil solution was arsenite (Fig. 1) and the uptake of arsenite by rice plant was higher than any other As species (Fig. 3), growing paddy rice in As-contaminated soil or by irrigating rice with As-contaminated water may cause elevated As concentration in the aerial plant parts. There are reports of elevated concentrations of As in rice straw because of application of As either in nutrient media or in soil (Marin et al., 1992, 1993a, 1993b; Xie and Huang, 1998). In our recent studies, we also observed a large accumulation of As by rice plants, with comparable root and straw concentrations of about 100 mg As kg^{-1} when rice was irrigated with a solution containing 8 mg As L^{-1} as arsenate (Abedin et al., 2001). In the same experiment, straw As concentration of 25 mg As kg^{-1} was found at the 2 mg As L^{-1} treatment (i.e. equivalent dose of reported highest contamination of Bangladesh groundwater). However, accumulation in rice grain was limited and was less than the maximum permissible limit of 1 mg kg^{-1} (National Food Authority, 1993). The presence of very high concentrations of As in rice straw could pose a potential health hazard to the cattle population because rice straw is used as cattle feed in Bangladesh and in other countries.

Our study has shown that DMAA and MMAA can be taken up by rice roots, albeit at a slow rate. Despite the presence of DMAA in the soil solution (Fig. 1), rice aerial tissue may contain smaller concentrations of DMAA because of lower uptake and restricted translocation of this species from root to other plant parts (Odanaka et al., 1987). This speculation regarding uptake and translocation of DMAA might be true because we found a small proportion of DMAA (0%–5% of total As) in the rice straw from our recent speciation study (Abedin et al., 2001). However, recent studies have also shown that DMAA could be a major component of total As in rice grain (Schoof et al., 1999; Heitkemper et al., 2001). MMAA uptake, on the other hand, is slightly higher than DMAA, but its presence in soil solution was low level (Fig. 1), and its restricted translocation from the root to shoot (Carbonell-Barrachina et al., 1998) may result in minimum translocation to aerial rice tissues. This speculation holds true from our rice straw speciation study (Abedin et al., 2001), where we did not detect any MMAA in the straw.

MATERIALS AND METHODS

Investigation of As Species in Soil Solution

Collection of Soil Solution

Soil solution was collected from a greenhouse pot experiment. Two 30-d-old seedlings of Aman paddy rice (*Oryza sativa*) var. BR11 were transplanted in 1-L plastic pots (with no perforation) packed with 1.1 kg of dry clay rich soil (Cruden Bay, NE Scotland). Arsenate was supplied as a solution of Na_2HAsO_4 and $7H_2O$ in distilled water in concentrations of 0 (control treatment), 0.0133 (1.0 mg As L^{-1}), 0.0266 (2.0 mg As L^{-1}), 0.0532 (4.0 mg As L^{-1}), and 0.1064 (8.0 mg As L^{-1}) mM to the experimental soil as required to maintain flooded paddy field conditions (i.e. saturation to permanent immersion of the soil under 3–4 cm of solution/distilled water, depending on treatment) throughout the life cycle of the plants. Phosphorus as $CaH_2PO_4.H_2O$. Water at 14.3 mg P kg^{-1} (equvalent to 30 kg ha^{-1}), K as KCl at 28.6 mg K kg^{-1} (equivalent to 60 kg ha^{-1}), and N as $CO(NH_2)_2$ at 76.3 mg N kg^{-1} (equivalent to 160 kg ha^{-1}) were supplied as solution (in distilled water) at the start of the experiment to ensure adequate mineral nutrition. Urea was applied to soil in four equal splits. The first application

was at transplantation, and subsequent applications at 30-d intervals. Application of all nutrient solutions and first application of arsenate treatment to dry soil was conducted before transplantation of rice seedlings. The experimental design was completely randomized with each treatment replicated three times. Daylight was supplemented with sodium lamps that were on for 8 h during the day; temperature fluctuation in the greenhouse was between 20°C and 35°C. Soil solutions collected by "Rhyzon soil solution sampler" from each treatment at the later stage of growth (about 9 weeks before harvesting the crop) were used to measure different As species. The "Rhizon soil solution sampler" is a special device produced by Rhizosphere Research Products (Wageningen, The Netherlands) to collect soil solutions with a minimum disturbance of the soil environment. It consists of: (a) a hydrophylic porous polymer tube of 2.3-mm diameter with a typical pore diameter 0.1 μm, having an internal stainless steel wire, to allow insertion into the soil; (b) a permanent connection to the soil surface; and (c) a luer-lock connector for attaching vacuum tubes or syringes to extract samples.

Analysis of As Species

Concentrations of As species in soil solution were measured by HPLC-inductively coupled plasma-mass spectrometry. A Hamilton PRP X-100 (250 mm $\times$ 4.1 mm, 10-μm column, Hamilton, Bonaduz, Switzerland) with a precolumn containing the same material was connected to a four-way Rheodyne valve (10-μL sample loop) and an HPLC pump (LKB, Uppsala). A solution of 30 mM H_3PO_4 set to pH 6.0 with NH_3 was used as a mobile phase with a flow rate of 1.0 mL min^{-1}, which allows a direct connection to a concentric nebulizer (Meriland C-Type) and a continuous transportation of the sample to the argon plasma of an ICP-mass spectrometer (Spectromass 2000, Spectro Analytical Instuments, Kleve, Germany). Standard plasma conditions were used. With a dwell time of 100 ms, the m/z 75 and 77 were monitored to check for possible ArCl interferences. Arsenite from $NaAsO_2$, arsenate from Na_2HAsO_4, $7H_2O$, MMAA from $CH_3AsO(ONa)_2$, and DMAA from $(CH_3)_2AsO(OH)$ were preserved as stock solutions at 1,000 mg As L^{-1}. Standard solutions (0–100 μg L^{-1}) were prepared fresh from stocks for calibration.

Kinetics of As Uptake

Growing Plants

One hundred milliliters of nutrient solution consisting of 0.2 mM $Ca(NO_3)_2$, 0.2 mM KNO_3, and 0.1 mM $MgSO_4$, $7H_2O$ was placed in to a plastic pot having 30 g of alkathene beads floated on the top. Four pregerminated rice seeds were then placed on the surface of the beads and the pots were placed in a greenhouse for 7 d. Eight Bangladeshi rice varieties were used for arsenite and arsenate high-affinity uptake experiments, of which four varieties (rice var. 1, BR11; rice var. 2, BR23; rice var. 3, BRRI Dhan 31; and rice var. 4, BRRI Dhan 33) are generally cultivated in the wet season (Aman) and four varieties (rice var. 5, BR1; rice var. 6, BRRI Dhan 26; rice var. 7, BRRI Dhan 27; and rice var. 8, Purbachi) are cultivated in the dry season (Boro). For other experiments, Aman season variety BR11 was used.

Uptake Kinetics

Roots of rice seedlings were excised at the basal node and replicate samples of excised roots were incubated in aerated nutrient solution (of the same composition used to grow seedlings) for 30 min at room temperature. Then the roots were incubated in aerated test solutions with different concentrations of arsenite/arsenate/DMAA/MMAA for 20 min. Test solution concentrations of arsenite, arsenate, MMAA, and DMAA for high-affinity uptake experiments ranged between 0 and 0.0532 mM, and for high- and low-affinity uptake experiments with arsenite and arsenate concentrations were 0 to 2.5 mM. The test solution contained 0.05 mM arsenite or arsenate for the phosphate competition experiment with phosphate concentrations ranging from 0 to 0.5 mM.

Stock solutions of arsenite, arsenate, DMAA, and MMAA were prepared from sodium arsenite ($NaAsO_2$), sodium arsenate (Na_2HAsO_4, $7H_2O$), dimetylarsinic acid [$(CH_3)_2AsO(OH)$], and disodium methane arsonate [$CH_3AsO(ONa)_2$], respectively. All test solutions contained 5.0 mM MES and 0.5 mM $Ca(NO_3)_2$ adjusted to pH 5 using KOH. In all experiments, after the termination of incubation in test solution, the roots were then rinsed in ice-cold phosphate solution containing 1 mM K_2HPO_4, 5 mM MES, and 0.5 mM $Ca(NO_3)_2$. The roots were then incubated for 10 min in the ice-cold phosphate solution of the same composition to remove the adsorbed As species from the root free space. Fresh weights of roots were then recorded.

Digestion and Analysis

The root samples were digested by 1 mL of concentrated Analar HNO_3. The digestion tubes were heated on a heating block at 180°C for 1 h and then at 200°C to evaporate the samples to dryness. The residue was taken up in 10 mL of 10% (w/v) HCl containing 10% (w/v) KI and 5% (w/v) ascorbic acid. As concentrations in the samples were then determined by hydride generation atomic absorption spectrophotometry.

Statistical Analysis

Data were analyzed by ANOVA using the computer package Minitab version 13 (State College, PA). Curve fitting was done using the computer package Sigma Plot (Jandel Scientific, Erkrath, Germany).

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